Bulletin of Mathematical Biology (2007) 69: 433–457
DOI 10.1007/s11538-006-9136-2

ORIGINAL ARTICLE

The Total Quasi-Steady-State Approximation for Fully Competitive Enzyme Reactions

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Received: 26 August 2005 / Accepted: 21 April 2006 / Published online: 19 July 2006

Abstract The validity of the Michaelis–Menten–Briggs–Haldane approximation for single enzyme reactions has recently been improved by the formalism of the total quasi-steady-state approximation. This approach is here extended to fully competitive systems, and a criterion for its validity is provided. We show that it extends the Michaelis–Menten–Briggs–Haldane approximation for such systems for a wide range of parameters very convincingly, and investigate special cases. It is demonstrated that our method is at least roughly valid in the case of identical affinities. The results presented should be useful for numerical simulations of many in vivo reactions.

Keywords Michaelis–Menten kinetics · Competitive substrates · Substrate–inhibitor system · Quasi-steady-state assumption

1. Introduction

Biochemistry in general and enzyme kinetics in particular have been heavily influenced by the model of biochemical reactions set forth by Henri (1901a,b, 1902) and Michaelis and Menten (1913), and further developed by Briggs and Haldane (1925). This formulation considers a reaction where a substrate S binds reversibly to an enzyme E to form a complex C. The complex can decay irreversibly to a

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product P and the enzyme, which is then free to bind another substrate molecule. This is summarized in the scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} E + P, \tag{1}$$

where k_1, k_{-1}, and k_2 are kinetic parameters (supposed constant) associated with the reaction rates.

Assuming that the complex concentration is approximately constant after a short transient phase leads to the usual Briggs–Haldane approximation (or *standard quasi-steady-state assumption* or *approximation* (standard QSSA, sQSSA)), which is valid when the enzyme concentration is much lower than either the substrate concentration or the Michaelis constant K_M (Segel, 1988). This is usually fulfilled for in vitro experiments, but sometimes breaks down in vivo (Straus and Goldstein, 1943; Sols and Marco, 1970; Albe et al., 1990). See Schnell and Maini (2003) for a nice and complete review of the kinetics and approximations of scheme (1).

The advantage of a quasi-steady-state approximation is that it reduces the dimensionality of the system, and thus speeds up numerical simulations greatly, especially for large networks as found in vivo. Moreover, while the kinetic constants in (1) are usually not known, finding the kinetic parameters characterizing the sQSSA is a standard procedure in in vitro biochemistry (Bisswanger, 2002). However, to simulate physiologically realistic in vivo scenarios, one faces the problem that the sQSSA might be invalid as mentioned above. Hence, even if the kinetic constants such as K_M are identical in vivo and in vitro, they need to be implemented in some other approximation which must be valid for the whole system and initial concentrations under investigation.

Approximations such as the *reverse QSSA* (rQSSA) (Segel and Slemrod, 1989; Schnell and Maini, 2000), which is valid for high enzyme concentrations, and the *total QSSA* (tQSSA) (Borghans et al., 1996; Tzafriri, 2003), which is valid for a broader range of parameters covering both high and low enzyme concentrations, have been introduced in the last two decades. Curiously, the rQSSA is equivalent to the *rapid-equilibrium approximation* proposed by Michaelis and Menten (1913), although their names are often connected to the sQSSA introduced by Briggs and Haldane (1925).

Tzafriri (2003) showed that the tQSSA is at least roughly valid for any set of parameters. Also, the tQSSA for reversible reactions has been studied (Tzafriri and Edelman, 2004), i.e., reactions of form (1), but where enzyme and product can recombine to form the complex.

These newer approximations have so far only been found for isolated reactions. However, in vivo the reactions are coupled in complex networks or cascades of intermediate, second messengers with successive reactions, competition between substrates, feedback loops, etc. Approximations of such scenarios have been carried out within the sQSSA scheme (Bisswanger, 2002), but often without a thorough investigation of the validity of the approximations. An exception is the case of fully competitive reactions (Segel, 1988; Schnell and Mendoza, 2000), i.e.,

reactions with competing substrates, also known as substrate–inhibitor systems,

$$\begin{aligned} S_1 + E &\underset{k_{-1}^{(1)}}{\overset{k_1^{(1)}}{\rightleftarrows}} C_1 \xrightarrow{k_2^{(1)}} E + P_1, \\ S_2 + E &\underset{k_{-1}^{(2)}}{\overset{k_1^{(2)}}{\rightleftarrows}} C_2 \xrightarrow{k_2^{(2)}} E + P_2, \end{aligned} \tag{2}$$

where S_i, C_i, and P_i represent substrate, enzyme–substrate complex, and product $i = 1, 2$, respectively. However, since the sQSSA cannot be expected to be valid in vivo, employing the tQSSA to these more complex situations would be beneficial.

This paper investigates the tQSSA for fully competitive reactions and is organized as follows. In Section 2 we recall the most important results in terms of quasi-steady-state approximations for a single reaction and for a fully competitive system. In Section 3 we introduce the tQSSA for a fully competitive system, discuss the timescales of the reactions, and introduce a sufficient condition for the validity of the tQSSA. Moreover, the form of the concentrations of the complexes C_i in the quasi-steady-state phase is investigated. In Section 4 we study the special case of identical affinities ($K_{\mathrm{M}}^{(1)} \approx K_{\mathrm{M}}^{(2)}$). The first-order approximation is obtained in terms of a perturbation parameter r, related to the characteristic constants of the system. Finally, a closed-form solution for the total substrate concentrations is obtained in this special case. In Section 5 the situation of very different affinities, for example reflecting a slow or fast competitive inhibitor, is studied. The corresponding approximations for the concentrations of C_i are found and used to obtain a general first-order approximation to the tQSSA for fully competitive reactions for any choice of $K_{\mathrm{M}}^{(i)}$, by means of Padé approximant techniques. In Section 6 we show numerically that for a very large range of parameters our tQSSA provides excellent fitting to the solutions of the full system, better than the sQSSA and the single-reaction tQSSA, and we discuss the obtained results.

2. Theoretical background

We recall briefly the mathematical description of the sQSSA for (1), using the same symbols for the concentrations of the reactants. The reaction (1) can be described by a system of two nonlinear ordinary differential equations. Assuming that the complex is in a quasi-steady-state leads to (Briggs and Haldane, 1925; Segel, 1988; Segel and Slemrod, 1989)

$$\frac{\mathrm{d}S}{\mathrm{d}t} \approx -\frac{V_{\max} S}{K_{\mathrm{M}} + S}, \quad S(0) = S_0, \tag{3}$$

Here $V_{\max} = k_2\, E_0 = k_2\, E(0)$ is the maximal reaction rate and $K_{\mathrm{M}} = \frac{k_{-1}+k_2}{k_1}$ is the Michaelis constant, identifying the substrate concentration giving the half-max reaction rate, i.e., K_{M} reflects the substrate affinity of the enzyme. This approximation is valid whenever (Segel, 1988; Segel and Slemrod, 1989),

$$\frac{E_0}{K_{\mathrm{M}} + S_0} \ll 1, \tag{4}$$

i.e., when the enzyme concentration is low with respect to either the Michaelis constant or to the substrate concentration.

The tQSSA (Borghans et al., 1996; Tzafriri, 2003) arises by changing to the total substrate originally introduced by Straus and Goldstein (1943) $\bar{S} = S + C$. Assuming that the complex is in a quasi-steady-state yields the tQSSA

$$\frac{\mathrm{d}\bar{S}}{\mathrm{d}t} \approx -k_2\, C_-(\bar{S}), \quad \bar{S}(0) = S_0, \tag{5}$$

where

$$C_-(\bar{S}) = \frac{(E_0 + K_{\mathrm{M}} + \bar{S}) - \sqrt{(E_0 + K_{\mathrm{M}} + \bar{S})^2 - 4E_0\bar{S}}}{2}. \tag{6}$$

Tzafriri (2003) showed that the tQSSA is valid whenever

$$\epsilon_{Tz} := \frac{K}{2S_0}\left(\frac{E_0 + K_{\mathrm{M}} + S_0}{\sqrt{(E_0 + K_{\mathrm{M}} + S_0)^2 - 4E_0S_0}} - 1\right) \ll 1, \quad K = \frac{k_2}{k_1}, \tag{7}$$

and that this is always roughly valid in the sense that

$$\epsilon_{Tz} \leq \frac{K}{4K_{\mathrm{M}}} \leq \frac{1}{4}. \tag{8}$$

The parameter K is known as the Van Slyke-Cullen constant. Tzafriri (2003) found

$$\frac{\mathrm{d}\bar{S}}{\mathrm{d}t} \approx -\frac{V_{\max}\bar{S}}{K_{\mathrm{M}} + E_0 + \bar{S}}, \quad \bar{S}(0) = S_0, \tag{9}$$

as a first-order approximation to (5). This expression (9) is identical to the formula obtained by Borghans et al. (1996) by means of a two-point Padé approximant technique (Baker, 1975), and it is valid at low enzyme concentrations (4) where it reduces to the sQSSA expression (3), but holds moreover, at low substrate concentrations $S_0 \ll E_0 + K_{\mathrm{M}}$ (Tzafriri, 2003). We wish to highlight the fundamental fact that performing the substitutions of S by $\bar{S}$ and of K_{M} by $K_{\mathrm{M}} + E_0$ one obtains a significantly improved sQSSA-like approximation with minimal effort.

The system (2) under investigation in this paper is governed by the coupled ODEs (Rubinow and Lebowitz, 1970; Segel, 1988; Schnell and Mendoza, 2000),

$i = 1, 2,$

$$\frac{\mathrm{d}S_i}{\mathrm{d}t} = -k_1^{(i)} E \cdot S_i + k_{-1}^{(i)} C_i, \qquad S_i(0) = S_{i,0}, \tag{10a}$$

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = k_1^{(i)} \left(E \cdot S_i - K_{\mathrm{M}}^{(i)} C_i\right), \quad C_i(0) = 0, \quad K_{\mathrm{M}}^{(i)} = \frac{k_{-1}^{(i)} + k_2^{(i)}}{k_1^{(i)}}. \tag{10b}$$

and the conservation laws

$$S_{i,0} = S_i + C_i + P_i, \quad i = 1, 2, \tag{11}$$

$$E_0 = E + C_1 + C_2. \tag{12}$$

The sQSSA of this system is (Rubinow and Lebowitz, 1970; Segel, 1988)

$$\frac{\mathrm{d}S_i}{\mathrm{d}t} = -\frac{k_2^{(i)} E_0 S_i}{K_{\mathrm{M}}^{(i)}(1 + S_j/K_{\mathrm{M}}^{(j)}) + S_i}, \quad S_i(0) = S_{i,0}, \quad i = 1, 2, \quad j \neq i, \tag{13}$$

which is valid when (Schnell and Mendoza, 2000)

$$\frac{E_0}{K_{\mathrm{M}}^{(i)}\left(1 + S_{j,0}/K_{\mathrm{M}}^{(j)}\right) + S_{i,0}} \ll 1, \quad i = 1, 2, \quad j \neq i. \tag{14}$$

Rubinow and Lebowitz (1970) showed that equations (13), $i = 1, 2$, can be uncoupled when introducing the parameter

$$\delta = \frac{k_2^{(1)} K_{\mathrm{M}}^{(2)}}{k_2^{(2)} K_{\mathrm{M}}^{(1)}},$$

which produces a measure of the competition. This parameter enters when dividing (13) for $i = 1$ with (13) for $i = 2$ and solving, which yields $S_1/S_{1,0} = (S_2/S_{2,0})^{\delta}$. Equations (13), $i = 1, 2$, then become

$$\frac{\mathrm{d}\bar{S}_1}{\mathrm{d}t} \approx -\frac{k_2^{(1)} E_0 \bar{S}_1}{K_{\mathrm{M}}^{(1)}\left(1 + S_{2,0}(\bar{S}_1/S_{1,0})^{1/\delta}/K_{\mathrm{M}}^{(2)}\right) + S_1}, \quad \bar{S}_1(0) = S_{1,0}, \tag{15a}$$

$$\frac{\mathrm{d}\bar{S}_2}{\mathrm{d}t} \approx -\frac{k_2^{(2)} E_0 \bar{S}_2}{K_{\mathrm{M}}^{(2)}\left(1 + S_{1,0}(\bar{S}_2/S_{2,0})^{\delta}/K_{\mathrm{M}}^{(1)}\right) + S_2}, \quad \bar{S}_2(0) = S_{2,0}. \tag{15b}$$

These expressions were used by Schnell and Mendoza (2000) to find analytic, closed-form solutions for the cases $\delta \approx 1$ and $\delta \ll 1$ using the so-called Lambert W-function.

3. Total quasi-steady-state approximation of the competitive system

Following Borghans et al. (1996), we introduce the total substrates

$$\bar{S}_i = S_i + C_i, \quad i = 1, 2, \tag{16}$$

and rewrite equations (10) in terms of these, obtaining the system of ODEs, $i = 1, 2$,

$$\frac{\mathrm{d}\bar{S}_i}{\mathrm{d}t} = -k_2^{(i)} C_i, \quad \bar{S}_i(0) = S_{i,0}, \tag{17a}$$

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = k_1^{(i)}\big((E_0 - C_1 - C_2)\cdot(\bar{S}_i - C_i) - K_{\mathrm{M}}^{(i)} C_i\big), \quad C_i(0) = 0. \tag{17b}$$

We require $0 < C_i < \bar{S}_i$, $i = 1, 2$, because of (16), and apply the quasi-steady-state assumption (Borghans et al., 1996; Tzafriri, 2003),

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} \approx 0, \quad i = 1, 2,$$

which is equivalent to the system

$$C_1 = E_0 - C_2\left(1 + \frac{K_{\mathrm{M}}^{(2)}}{\bar{S}_2 - C_2}\right), \tag{18a}$$

$$C_2 = E_0 - C_1\left(1 + \frac{K_{\mathrm{M}}^{(1)}}{\bar{S}_1 - C_1}\right), \tag{18b}$$

which should hold for any time t after the initial transient. From this system, it follows that $C_i < E_0$, $i = 1, 2$, in agreement with (12). As shown in Appendix A, the system (18) has a unique solution with $0 < C_i < \min\{\bar{S}_i, E_0\}$. For C_1 it is given by finding the appropriate root of the third-degree polynomial

$$\begin{aligned}\psi_1(C_1) = &-\big(K_{\mathrm{M}}^{(1)} - K_{\mathrm{M}}^{(2)}\big)C_1^3 \\ &+\big[\big(E_0 + K_{\mathrm{M}}^{(1)} + \bar{S}_1\big)\big(K_{\mathrm{M}}^{(1)} - K_{\mathrm{M}}^{(2)}\big) - \big(\bar{S}_1 K_{\mathrm{M}}^{(2)} + \bar{S}_2 K_{\mathrm{M}}^{(1)}\big)\big]C_1^2 \\ &+\big[-E_0\big(K_{\mathrm{M}}^{(1)} - K_{\mathrm{M}}^{(2)}\big) + \big(\bar{S}_1 K_{\mathrm{M}}^{(2)} + \bar{S}_2 K_{\mathrm{M}}^{(1)}\big) + K_{\mathrm{M}}^{(2)}\big(E_0 + K_{\mathrm{M}}^{(1)}\big)\big]\bar{S}_1 C_1 \\ &- E_0 K_{\mathrm{M}}^{(2)} \bar{S}_1^2.\end{aligned} \tag{19}$$

When $K_{\mathrm{M}}^{(1)} = K_{\mathrm{M}}^{(2)} = K_{\mathrm{M}}$, ψ_1 becomes a second-degree polynomial, and the root is given by

$$C_1 = \frac{\bar{S}_1(\bar{S}_1 + \bar{S}_2 + K_{\mathrm{M}} + E_0)}{2(\bar{S}_1 + \bar{S}_2)}\left(1 - \sqrt{1 - \frac{4E_0(\bar{S}_1 + \bar{S}_2)}{(\bar{S}_1 + \bar{S}_2 + K_{\mathrm{M}} + E_0)^2}}\right). \tag{20}$$

An analogous polynomial ψ_2 for C_2 can be found by interchanging the indexes 1 and 2 in (19), because of the symmetry of the system (17), and C_2 is again found as the appropriate root.

3.1. *Validity of the tQSSA*

We expect that after a short transient phase the complex concentrations equal at any time t the instantaneous quasi-steady-state concentrations, $C_i(t) = C_i(\bar{S}_1(t), \bar{S}_2(t))$, given by the roots in the respective polynomials as discussed above. Then the evolution of the system can be studied by means of the tQSSA

$$\frac{\mathrm{d}\bar{S}_i}{\mathrm{d}t} \approx -k_2^{(i)} C_i(\bar{S}_1, \bar{S}_2), \quad \bar{S}_i(0) = S_{i,0}. \tag{21}$$

Segel (1988) proposed the following two criteria for the validity of a QSSA.

(i) The timescale for the complex(es) during the transient phase, t_C, should be much smaller than the timescale for changes in the substrate(s) in the beginning of the quasi-steady-state phase, t_S.
(ii) The substrate(s) should be nearly constant during the transient phase.

In our case, (ii) can be translated to (Segel, 1988; Tzafriri, 2003)

$$\frac{S_{i,0} - \bar{S}_i}{S_{i,0}} \leq \frac{t_C}{S_{i,0}} \max \left| \frac{\mathrm{d}\bar{S}_i}{\mathrm{d}t} \right| = \frac{k_2^{(i)} t_C}{S_{i,0}} C_i(S_{1,0}, S_{2,0}) \ll 1, \quad i = 1, 2, \tag{22}$$

where the maximum is taken over the transient phase, i.e., with $\bar{S}_i \approx S_{i,0}$. Since C_i is increasing during the transient phase, the maximum is given by $k_2^{(i)} C_i(S_{1,0}, S_{2,0})$.

The substrate timescale (Segel, 1988; Tzafriri, 2003) is estimated from (21) to be

$$t_{\bar{S}_i} \approx \frac{S_{i,0}}{k_2^{(i)} C_i(S_{1,0}, S_{2,0})}, \tag{23}$$

and we see that (22) translates into (i), i.e.,

$$\max_{i=1,2} \frac{t_C}{t_{\bar{S}_i}} = \frac{\max\{t_{C_1}, t_{C_2}\}}{\min\{t_{\bar{S}_1}, t_{\bar{S}_2}\}} \ll 1. \tag{24}$$

The timescale for the complexes is estimated following Borghans et al. (1996):

$$t_{C_i} \approx \frac{C_i(S_{1,0}, S_{2,0})}{\max \left| \frac{\mathrm{d}C_i}{\mathrm{d}t} \right|} = \frac{C_i(S_{1,0}, S_{2,0})}{k_1^{(i)} E_0 S_{i,0}}, \tag{25}$$

where the maximum is again taken during the transient phase. The timescale for the transient phase is then the maximum of the two individual scales; we want

both complexes to be in the quasi-steady-state at the end of the transient phase, and both substrates to be nearly constant during it.

Hence, we propose the following *sufficient* condition for the validity of our tQSSA (21),

$$\epsilon := \max_{i=1,2}\left(\frac{k_2^{(i)}C_i(S_{1,0},S_{2,0})}{S_{i,0}}\right)\max_{i=1,2}\left(\frac{C_i(S_{1,0},S_{2,0})}{k_1^{(i)}E_0S_{i,0}}\right) \ll 1. \tag{26}$$

Whenever the two maxima occur for the same i, (26) simplifies to

$$\bar{\epsilon} = \max_{i=1,2}\left(\frac{K_i}{E_0}\left(\frac{C_i(S_{1,0},S_{2,0})}{S_{i,0}}\right)^2\right) \ll 1, \tag{27}$$

where we introduced the Van Slyke–Cullen constants $K_i = k_2^{(i)}/k_1^{(i)}$.

4. Identical affinity, $K_M^{(1)} \approx K_M^{(2)}$

When $K_M^{(1)} \neq K_M^{(2)}$ the roots of ψ_1 are given by a very complicated formula in contrast to the formula (20) when $K_M^{(1)} \approx K_M^{(2)} = K_M$. To deepen our understanding of the problem we follow this latter case further. It should be noted that the situation is biologically realistic, for example, for bacterial carbohydrate sulfotransferase (NodST) with chitotriose and chitopentose as competitive substrates (Pi and Leary, 2004), for IκB kinase (IKK-2) phosphorylation of IκBα and p65, which is of importance in inflammatory diseases (Kishore et al., 2003) and for the double phosphorylation of MAPK by MAPKK (Huang and Ferrell, 1996; Bhalla and Iyengar, 1999; Kholodenko, 2000). Following Tzafriri (2003) we let

$$r(X) = \frac{4E_0X}{(X+K_M+E_0)^2},$$

where X is some unspecified substrate concentration. Then we can rewrite (20) as

$$C_1(\bar{S}_1,\bar{S}_2) = \frac{\bar{S}_1(\bar{S}_1+\bar{S}_2+K_M+E_0)}{2(\bar{S}_1+\bar{S}_2)}(1-\sqrt{1-r(\bar{S}_1+\bar{S}_2)}). \tag{28}$$

Setting

$$K = \frac{\max\{k_2^{(1)},k_2^{(2)}\}}{\min\{k_1^{(1)},k_1^{(2)}\}}, \quad S_0 = S_{1,0}+S_{2,0}, \quad r_0 = r(S_0), \tag{29}$$

we get from (26) and (28) that

$$\begin{aligned}\epsilon &= \frac{K}{E_0}\left(\frac{S_0+K_{\mathrm{M}}+E_0}{2S_0}(1-\sqrt{1-r_0})\right)^2\\ &= \frac{K}{S_0}\frac{(1-\sqrt{1-r_0})^2}{1-(1-r_0)}\\ &= \frac{K}{S_0}\frac{1-\sqrt{1-r_0}}{2}\frac{2}{1+\sqrt{1-r_0}}\\ &\leq \frac{K}{2S_0}\frac{1-\sqrt{1-r_0}}{\sqrt{1-r_0}}\\ &= \epsilon_{Tz},\end{aligned}$$

where ϵ_{Tz} is the expression from (7). Let us remark that the constant K in (29) is different from the Van Slyke–Cullen constant appearing in a single reaction. We can now use the result (8) to get

$$\epsilon \leq \epsilon_{Tz} \leq \frac{K}{4K_{\mathrm{M}}}. \tag{30}$$

Inequality (30) tells us that, for identical affinities, we have that, the smaller the ratio K/K_{M}, the better the tQSSA approximation (21). If $K = k_2^{(i)}/k_1^{(i)}$ (same i), then $K \leq K_{\mathrm{M}}$, and hence, $\epsilon \leq \frac{1}{4}$, such that in this case the tQSSA (21) is at least roughly valid. However, this is not necessarily true if $K = k_2^{(i)}/k_1^{(j)}$ $(j \neq i)$.

4.1. First-order tQSSA for identical affinities

Developing (28) in r yields

$$C_1 = \frac{E_0\bar{S}_1}{\bar{S}_1+\bar{S}_2+K_{\mathrm{M}}+E_0} + O(r^2). \tag{31}$$

In this case (compare with Borghans et al. (1996))

$$\epsilon = \frac{KE_0}{(S_{1,0}+S_{2,0}+K_{\mathrm{M}}+E_0)^2} + O(r^2), \quad K = \frac{\max\{k_2^{(1)}, k_2^{(2)}\}}{\min\{k_1^{(1)}, k_1^{(2)}\}}. \tag{32}$$

When $r \ll 1$ *and* the tQSSA is valid ($\epsilon \ll 1$), we obtain the *first-order* tQSSA (with respect to r) for competing substrates with identical affinity

$$\frac{\mathrm{d}\bar{S}_i}{\mathrm{d}t} \approx -\frac{k_2^{(i)}E_0\bar{S}_i}{\bar{S}_1+\bar{S}_2+K_{\mathrm{M}}+E_0}, \quad \bar{S}_i(0) = S_{i,0}, \quad i = 1, 2. \tag{33}$$

The sufficient conditions for $r \ll 1$ from Tzafriri (2003) translate into either of

$$S_{1,0} + S_{2,0} + K_M \gg E_0, \tag{34}$$

$$E_0 + K_M \gg S_{1,0} + S_{2,0}. \tag{35}$$

The condition (34) also guarantees $\epsilon \ll 1$ because of (32), unless $K \gg K_M + S_{1,0} + S_{2,0}$. As noted above, $K \leq K_M$ if $K = k_2^{(i)}/k_1^{(i)}$ (same i), and then indeed $\epsilon \ll 1$.

However, (35) does not imply $\epsilon \ll 1$ but must be accompanied by $K \ll K_M$, in which case (30) guarantees $\epsilon \ll 0.25$. When $K \gtrapprox K_M$ we must require $E_0 \gg K$ such that (32) yields $\epsilon \ll 1$, and in this case (35) simplifies to $E_0 \gg S_{1,0} + S_{2,0}$. In summary, any of the following conditions imply the validity of the first-order tQSSA for identical affinities:

$$E_0 \ll S_{1,0} + S_{2,0} + K_M, \quad \text{and} \quad K \lessapprox K_M + S_{1,0} + S_{2,0} \tag{36}$$

$$E_0 + K_M \gg S_{1,0} + S_{2,0}, \quad \text{and} \quad K \ll K_M, \tag{37}$$

$$E_0 \gg S_{1,0} + S_{2,0}, \quad \text{and} \quad E_0 \gg K \gtrapprox K_M. \tag{38}$$

Neglecting E_0 in the denominator in (33) we obtain again the sQSSA of competing substrates with identical affinities (see (13) with $K_M^{(1)} = K_M^{(2)} = K_M$). This is valid when (36) holds, as seen from (14). On the other hand, when (37) or (38) is fulfilled, (33) does not reduce to the sQSSA (13). Hence, (37) or (38) extend the parameter region where (33) is valid.

4.2. Uncoupled equations and closed-form solutions

When $K_M^{(1)} \approx K_M^{(2)}$ as above, the two equations given by (21) can be uncoupled (Rubinow and Lebowitz, 1970; Segel, 1988; Schnell and Mendoza, 2000) by dividing one by the other and using (20), leading to

$$\frac{d\bar{S}_1}{d\bar{S}_2} = \frac{k_2^{(1)}}{k_2^{(2)}} \frac{\bar{S}_1}{\bar{S}_2},$$

such that

$$\frac{\bar{S}_1}{S_{1,0}} = \left(\frac{\bar{S}_2}{S_{2,0}}\right)^{\delta}, \quad \delta = \frac{k_2^{(1)}}{k_2^{(2)}}. \tag{39}$$

This relation can then be used to eliminate $\bar{S}_2$ in (20) and, similarly, eliminate $\bar{S}_1$ in the expression for C_2. It also shows that when $\delta = 1$, i.e., $k_2^{(1)} = k_2^{(2)} = k_{cat}$, the two substrates behave identically with the only difference given by their initial concentrations. This can also be observed from (21) with (20) inserted.

In the following we assume that the first-order tQSSA (33) holds. Using (39) we write (33) as

$$\frac{\mathrm{d}\bar{S}_1}{\mathrm{d}t} \approx -\frac{k_2^{(1)} E_0 \bar{S}_1}{\bar{S}_1 + S_{2,0}(\bar{S}_1/S_{1,0})^{1/\delta} + K_\mathrm{M} + E_0}, \quad \bar{S}_1(0) = S_{1,0}, \tag{40}$$

$$\frac{\mathrm{d}\bar{S}_2}{\mathrm{d}t} \approx -\frac{k_2^{(2)} E_0 \bar{S}_2}{\bar{S}_2 + S_{1,0}(\bar{S}_2/S_{2,0})^{\delta} + K_\mathrm{M} + E_0}, \quad \bar{S}_2(0) = S_{2,0}. \tag{41}$$

These equations are identical to (15) studied by Schnell and Mendoza (2000) setting $K_\mathrm{M}^{(1)} = K_\mathrm{M}^{(2)}$ and applying the substitution $K_\mathrm{M} \to K_\mathrm{M} + E_0$. Hence, the same techniques can be used to find closed-form solutions.

When $\delta = 1$, (40) and (41) are identical except from the initial conditions and hence the two substrates develop identically, as observed above. The solution is given in closed form by

$$\bar{S}_i(t) \approx S_{i,0} \frac{K_\mathrm{M} + E_0}{S_{1,0} + S_{2,0}} \cdot W\left(\frac{S_{1,0} + S_{2,0}}{K_\mathrm{M} + E_0} \exp\left(\frac{S_{1,0} + S_{2,0} - k_\mathrm{cat} E_0 t}{K_\mathrm{M} + E_0}\right)\right), \tag{42}$$

where W is the Lambert W-function introduced in enzyme kinetics by Schnell and Mendoza (1997). It is defined as the real valued solution to $W(x)\exp(W(x)) = x$.

The case when $\delta \ll 1$ corresponds to a slow (resp. fast) competitor, when $\bar{S}_1$ is regarded as the competitor (resp. substrate) and $\bar{S}_2$ as the substrate (resp. competitor). The closed-form solution can again be found following Schnell and Mendoza (2000), giving

$$\bar{S}_2(t) \approx (S_{1,0} + K_\mathrm{M} + E_0) W\left(\frac{S_{2,0}}{S_{1,0} + K_\mathrm{M} + E_0} \exp\left(\frac{S_{2,0} - k_2^{(2)} E_0 t}{S_{1,0} + K_\mathrm{M} + E_0}\right)\right),$$

$$\bar{S}_1(t) \approx S_{1,0}\left(\frac{\bar{S}_2(t)}{S_{2,0}}\right)^{\delta}. \tag{43}$$

At low substrate concentrations (see formula (35)), the argument of W in (42) and (43) is small and the approximation $W(x) \approx x$ holds. Hence, for both $\delta \approx 1$ and $\delta \ll 1$ we find after some algebra

$$\bar{S}_i(t) \approx S_{i,0} \exp\left(-\frac{k_2^{(i)} E_0}{K_\mathrm{M} + E_0}\, t\right), \tag{44}$$

which is identical to the expression for the tQSSA of an isolated reaction (Tzafriri, 2003). Hence, the two substrates behave completely independently as if they were isolated. The same result is found directly by neglecting $\bar{S}_1 + \bar{S}_2$ in the denominator of (33). This is due to either K_M or the enzyme concentration being much greater than the substrate concentrations, such that the fraction of free enzyme in the system is near to unity. Schnell and Mendoza (1997a,b) studied this scenario for the sQSSA.

5. $K_M^{(1)} \gg K_M^{(2)}$ and the general first-order approximation

We now turn to the case of very different affinities as stated by $K_M^{(1)} \gg K_M^{(2)}$. To investigate this situation closer we perform a perturbation around $K_M^{(2)} = 0$. When $K_M^{(2)} = 0$, we see from (17b) that in the quasi-steady-state, $C_2 \approx \bar{S}_2$ or $E_0 - C_1 - C_2 \approx 0$. In the former case, $C_2 \approx S_{2,0}$ since $k_2^{(2)} = 0$. In the latter case, again from (17b), but now for $i = 1$, it follows that $C_1 \approx 0$, $C_2 \approx E_0$. Since $C_2 \leq \min\{S_{2,0}, E_0\}$ we get that, when $K_M^{(2)} = 0$,

$$C_2 \approx \min\{S_{2,0}, E_0\}. \tag{45}$$

We study these two cases independently, and by means of their corresponding solutions we build a two-point Padé approximant (TPPA) (Baker, 1975) in $\eta = S_{2,0}/E_0$ developed around $\eta = 0$ ($E_0 \gg S_{2,0}$) and $\eta = \infty$ ($E_0 \ll S_{2,0}$).

When $E_0 \gg S_{2,0}$ (i.e., $\eta \ll 1$), we expect that after the transient phase the system evolves as two independent reactions: S_2 binds with a part of the enzyme during the transient phase as seen from (45), $C_2 \approx S_{2,0}$, leaving $E_0^* = E_0 - S_{2,0}$ to react with S_1. Hence, we obtain from (5) and (9)

$$\begin{aligned} \frac{\mathrm{d}\bar{S}_1}{\mathrm{d}t} &\approx -k_2^{(1)} \frac{(E_0^* + K_M^{(1)} + \bar{S}_1) - \sqrt{(E_0^* + K_M^{(1)} + \bar{S}_1)^2 - 4E_0^*\bar{S}_1}}{2} \\ &\approx -\frac{k_2^{(1)} E_0^* \bar{S}_1}{K_M^{(1)} + E_0^* + \bar{S}_1}. \end{aligned} \tag{46}$$

Note that here $K_M^{(2)} = 0$, such that (18a) is not valid. The solution can also be found by setting $C_2 = S_{2,0}$ in (17b) with $i = 1$.

In the case when $0 < K_M^{(2)} \ll K_M^{(1)}$, we neglect terms involving $K_M^{(2)}$ in (19) and obtain that C_1 should satisfy

$$\left(C_1^2 - \left(E_0 + K_M^{(1)} + \bar{S}_1 - \bar{S}_2\right)C_1 + (E_0 - \bar{S}_2)\bar{S}_1\right) \cdot C_1 = 0.$$

Since $C_2 \leq S_{2,0} < E_0$, $C_1 = 0$ is in contradiction with (18b). Thus, C_1 solves the second-degree polynomial which is exactly the polynomial given from the tQSSA for an isolated reaction, i.e., $\bar{S}_1$ follows again (46) but now with $E_0^* = E_0 - \bar{S}_2$.

We now turn to the case when $C_2 \approx E_0 \ll S_{2,0}$ (i.e., $\eta \gg 1$). Recall that this is the case of the usual in vitro experiments. From the conservation law (12) $C_1 \approx 0$. We expand $\psi_1/K_M^{(1)}$ in terms of the small parameter $\rho = K_M^{(2)}/K_M^{(1)}$ and find that the first-order term for the root is given by

$$C_1 = \rho \frac{E_0 \bar{S}_1}{\bar{S}_2 - E_0} = \frac{K_M^{(2)}}{K_M^{(1)}} \times \frac{E_0 \bar{S}_1}{\bar{S}_2 - E_0}. \tag{47}$$

Using (47) for $1/\eta \approx 0$, and the first-order approximation (46) for $\eta \approx 0$, the TPPA in $\eta = \bar{S}_2/E_0$ is

$$C_1 = \frac{E_0\bar{S}_1}{K_M^{(1)} + \bar{S}_1 + E_0 + \bar{S}_2/\rho} = \frac{E_0\bar{S}_1}{K_M^{(1)}\left(1 + \bar{S}_2/K_M^{(2)}\right) + \bar{S}_1 + E_0}. \tag{48}$$

Plugging (48) into (21) (for $i = 1$) yields then

$$\frac{d\bar{S}_i}{dt} = -\frac{k_2^{(i)} E_0 \bar{S}_i}{K_M^{(i)}\left(1 + \bar{S}_j/K_M^{(j)}\right) + \bar{S}_i + E_0}, \quad \bar{S}_i(0) = S_{i,0}, \quad j \neq i. \tag{49}$$

where $i = 1$, $j = 2$. Similar computations can be performed for C_2, when $K_M^{(1)} \ll K_M^{(2)}$, yielding the same equation (47), where $i = 2$, $j = 1$.

The two approximations hold for two different regions of parameter space, $K_M^{(1)} \gg K_M^{(2)}$ and $K_M^{(1)} \ll K_M^{(2)}$, respectively. However, let us observe that they reduce not only to the case of identical affinities, (33), for $K_M^{(1)} = K_M^{(2)}$, but also to the sQSSA (13) whenever this approximation holds as guaranteed by (14), and to the single-reaction first-order tQSSA (9) when $\bar{S}_j/K_M^{(j)}$ can be neglected.

Motivated by this and further encouraged by numerical simulations (see the following section), we propose the expression (49) (for $i = 1, 2$) as the general first-order approximation to the tQSSA for fully competitive reactions.

Although not strictly theoretically founded, the above considerations using the TPPA can be seen as the motivation for the formula. However, as shown in Appendix B, we can indeed expect C_1 from (48) (and C_2 in the corresponding expression) to be a good approximation to the true root of ψ_1 (ψ_2 for C_2) when either (14) holds, or when

$$K_M^{(i)} \gg S_{1,0} + S_{2,0} \quad \text{or} \quad E_0 \gg K_M^{(i)}\left(1 + S_{j,0}/K_M^{(j)}\right) + S_{i,0}, \quad i = 1, 2, \quad j \neq i.$$

Hence, (49) extends both the sQSSA (13) as well as the single-reaction tQSSA (5).

The considerations in Appendix B tell us only when the first-order tQSSA (49) is a good approximation of the full tQSSA (21), but neither might be a good representation of the full system. To assure that, ϵ must be small.

When (48) approximates the full tQSSA we have from (26)

$$\begin{aligned}
\epsilon &= \max_{i=1,2} \frac{k_2^{(i)} E_0}{\tilde{K}_{M,0}^{(i)} + S_{i,0} + E_0} \max_{i=1,2} \frac{1}{k_1^{(i)}\left(\tilde{K}_{M,0}^{(i)} + S_{i,0} + E_0\right)} \\
&\leq K \max_{i=1,2} \frac{E_0}{\tilde{K}_{M,0}^{(i)} + S_{i,0} + E_0} \max_{i=1,2} \frac{1}{\tilde{K}_{M,0}^{(i)} + S_{i,0} + E_0} \\
&= \max_{i=1,2} \frac{K E_0}{\left(\tilde{K}_{M,0}^{(i)} + S_{i,0} + E_0\right)^2}
\end{aligned} \tag{50}$$

where

$$K = \frac{\max\{k_2^{(1)}, k_2^{(2)}\}}{\min\{k_1^{(1)}, k_1^{(2)}\}}, \quad \tilde{K}_{\mathrm{M},0}^{(i)} = K_{\mathrm{M}}^{(i)}\left(1 + S_{j,0}/K_{\mathrm{M}}^{(j)}\right), \quad i = 1, 2, \quad j \neq i. \tag{51}$$

The above considerations yield that either one of the following conditions guarantees the validity of the first-order approximation (49) ($i = 1, 2$):

$$E_0 \ll S_{i,0} + \tilde{K}_{\mathrm{M},0}^{(i)}, \quad \text{and} \quad K \lessapprox \tilde{K}_{\mathrm{M},0}^{(i)} + S_{i,0}, \tag{52}$$

$$K_{\mathrm{M}}^{(i)} \gg S_{1,0} + S_{2,0}, \quad \text{and} \quad K \ll \tilde{K}_{\mathrm{M},0}^{(i)}, \tag{53}$$

$$K_{\mathrm{M}}^{(i)} \gg S_{1,0} + S_{2,0}, \quad \text{and} \quad E_0 \gg K \lessapprox \tilde{K}_{\mathrm{M},0}^{(i)}, \tag{54}$$

$$E_0 \gg \tilde{K}_{\mathrm{M},0}^{(i)} + S_{i,0}, \quad \text{and} \quad E_0 \gg K. \tag{55}$$

6. Numerical results and discussion

In vivo reactions are usually modeled by ordinary differential equations using the concentrations of the involved biochemical species. This idea has been questioned to hold for a low number of involved molecules or in a crowded environment, where stochastic methods should be used (Turner et al., 2004). However, in a comparison between the deterministic (sQSSA) and stochastic approaches it was found that the two approaches agreed reasonably well for as few as 100 molecules, and thus it was concluded that intracellular enzyme reactions as a rule are well described by the deterministic approach using concentrations (Turner et al., 2004).

The introduction of the recent total quasi-steady-state approximation (tQSSA) (Borghans et al., 1996; Tzafriri, 2003) is motivated by the need to extend the sQSSA to situations where the enzyme concentration is comparable to or greater than both the substrate concentration and the Michaelis constant. Albe et al. (1990) found that the enzyme concentration was greater than the corresponding substrate concentration in 12% of investigated substrate–enzyme pairs, and the two concentrations comparable were in other 13%, such that the enzyme concentration was more than 10 times lower than the substrate concentration in only 75% of the substrate–enzyme pairs. For comparison, Stayton and Fromm (1979) found that for the sQSSA to hold, one needs that the enzyme concentration is at least 100 times lower than the substrate concentration. However, it should be noted that we find excellent fits for the competitive sQSSA, when the enzyme to substrate ratio is 0.1 (Fig. 2A). Nonetheless, even though the majority of the enzyme-reaction pairs investigated by Albe et al. (1990) could be expected to be well approximated by the sQSSA, a significant number cannot, and such an approximation would break down in particular in the glycolytic pathway (Albe et al., 1990). It seems reasonable to expect that the same conclusion might hold for other pathways. And even in pathways where a few steps are badly approximated by the sQSSA, the

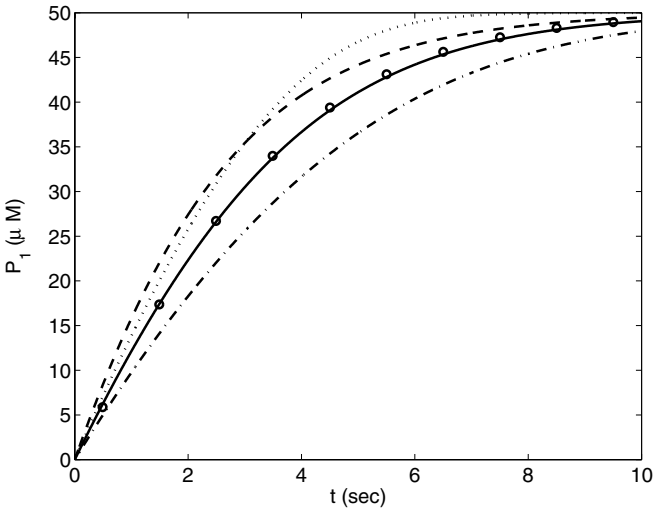

Fig. 1 The competitive tQSSA ((21), *full curve*) approximates the full system ((10), *circles*) very well ($R^2 = 0.9997$), also when both the competitive sQSSA ((13), *dotted curve*, $R^2 = 0.9321$) and the single-reaction tQSSA ((5), *dashed curve*, $R^2 = 0.9531$) do not. However, in this case we cannot obtain that the competitive first-order approximation ((49), *dash-dotted curve*, $R^2 = 0.9298$) is good. Parameters (based on Pi and Leary (2004)): $K_M^{(1)} = 23\,\mu$M, $K_M^{(2)} = 25\,\mu$M, $k_2^{(1)} = 42\,\text{min}^{-1}$, $k_2^{(2)} = 25\,\text{min}^{-1}$, $S_{1,0} = S_{2,0} = E_0 = 50\,\mu$M ($\epsilon = 0.022$). To fix $k_j^{(i)}$ we have used the constraint $k_{-1}^{(i)} = 4k_2^{(i)}$ (Bhalla and Iyengar, 1999).

inappropriate use of the sQSSA through out the pathway could yield erroneous predictions of the overall behavior (Pedersen et al., 2006).

In the present manuscript we have extended the total quasi-steady-state assumption to competing substrates, investigating its validity and deepening some special cases. As seen in Fig. 1, the approximation (21) is indeed excellent as long as ϵ is small. This figure is based on the data from Pi and Leary (2004) for carbohydrate sulfotransferase (NodST) with chitopentaose and chitotriose as competing substrates. Of importance, our approximation (21) captures the competition as does the sQSSA (13) and in contrast with the single-reaction tQSSA (5), but also at intermediate or high enzyme concentrations where the sQSSA (13) does not hold anymore (Fig. 1). However, when the competition can be neglected due to, e.g., low substrate concentrations, the single-reaction tQSSA (5) does indeed estimate the full system well (see, e.g., Fig. 2, panels B and C).

A crucial step of our analysis is finding the roots of the third-degree polynomials ψ_i. Although we have shown that there is exactly one physically possible root for each complex, and that there exists, e.g., Cardano's formula for this root, the formula is hard to interpret and even to implement. We have used a differential-algebraic equations (DAE) approach, i.e., finding the roots numerically. Such a DAE approach is easier to implement than using the closed form for the root, but increases the time needed for computations.

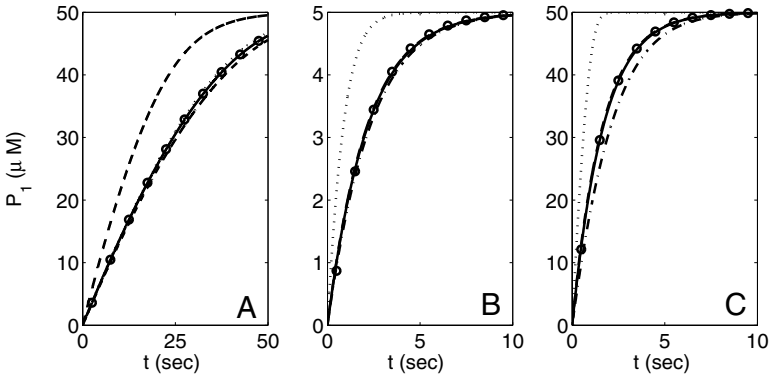

Fig. 2 The first-order approximation (*dash-dot curve*) coincides with the competitive sQSSA (*dotted curve*) when it is valid (panel A), and with the single-reaction tQSSA (*dashed curve*) when the competition is negligible (panel B). However, at high enzyme concentrations the single-reaction tQSSA is often a better approximation than the first-order tQSSA (panel C). Parameters are as in Fig. 1, except: In A: $E_0 = 5\,\mu$M ($\epsilon = 0.0027$). R^2 values (in the following order [*competitive tQSSA* (21), *competitive sQSSA* (13), *single-reaction tQSSA* (5), *competitive first-order approximation* (49)]): [1.0000, 0.9994, 0.6485, 0.9967]. In B: $S_{1,0} = S_{2,0} = 5\,\mu$M ($\epsilon = 0.0694$). R^2 values: [0.9985, 0.5113, 0.9980, 0.9969]. In C: $E_0 = 200\,\mu$M ($\epsilon = 0.029$). R^2 values: [0.9998, 0.4736, 0.9992, 0.9637].

These problems can partly be resolved by using approximations of the roots of ψ_i. Compared to the full solution, such an approximation should preferably be easier to interpret and to relate to previously known formulas. Furthermore, it should be clearly stated when it is valid. We found a first-order approximation (49), which is valid when the sQSSA approach (13) is as stated in (52), and in this case they coincide (Fig. 2A). Moreover, it is valid for high $K_{\mathrm{M}}^{(i)}$ values (conditions (53) and (54)), where it reduces to the single-reaction first-order approximation (5), see Fig. 2B. Hence, it extends these two approximations beyond the regions where they are known to hold. Finally, the first-order approximation is valid at high enzyme concentrations (55), but it is not always accurate if the enzyme concentration is only moderately high. In this case, the single-reaction tQSSA (5) is often a better approximation (Fig. 2C).

The case of very different affinities was used to derive the first-order approximation (49) using a two-point Pad approximant. However, it is of its own biological interest as seen for example from the data by Pi and Leary (2004) for carbohydrate sulfotransferase (NodST) with chitopentaose as substrate ($K_{\mathrm{M}}^{(1)} = 23\ \mu$M), and chitobiose as a competing substrate ($K_{\mathrm{M}}^{(2)} = 240\ \mu$M). Figure 3 shows that the full tQSSA (21) approximates the full system very well also in this specific example of $K_{\mathrm{M}}^{(1)} \ll K_{\mathrm{M}}^{(2)}$, even when all other approximations fail. For P_2 (Fig. 3B) it is seen that the sQSSA (13) overestimates the transient phase in which mainly P_1 (Fig. 3A) is produced, where after it is accelerated, such that the overall behavior is not only quantitatively, but also qualitatively wrongly estimated in this example. Curiously, the single-reaction tQSSA (5) estimates P_1 well. The reason is the low degree of competition felt by the first reaction as seen from $\tilde{K}_{\mathrm{M},0}^{(1)} \approx 2K_{\mathrm{M}}^{(1)} \ll E_0$, so

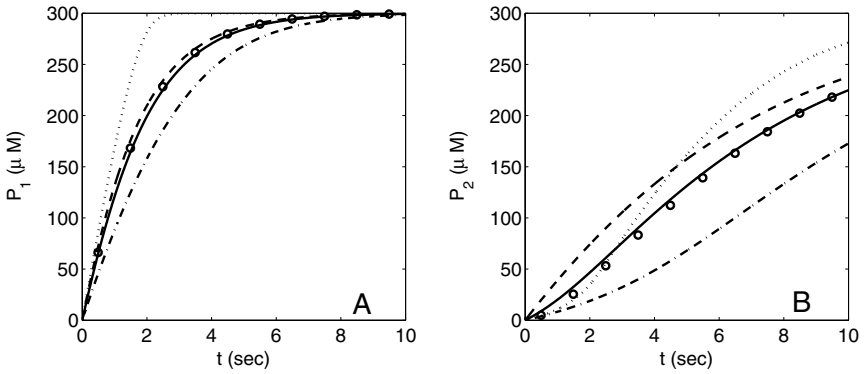

Fig. 3 Also $K_M^{(1)} \ll K_M^{(2)}$ is captured well by the competitive tQSSA (21). Legends and kinetic parameters for reaction 1 are as in Fig. 1, but $K_M^{(2)} = 240\,\mu$M, $k_2(2) = 19.5\,\text{min}^{-1}$, and $S_{1,0} = S_{2,0} = E_0 = 300\,\mu$M ($\epsilon = 0.0387$). R^2 values; panel A: [0.9992, 0.7778, 0.9974, 0.8858]; panel B: [0.9954, 0.7641, 0.8556, 0.5321].

both $\tilde{K}_{M,0}^{(1)}$ and $K_M^{(1)}$ are negligible compared to E_0. This is not true for the second reaction as illustrated in Fig. 3B.

The fractional errors associated with the different approximations are estimated as $\sqrt{1-R^2}$, where $R^2 = 1 - \sum_i (y_i - \hat{y}_i)^2 / \sum_i (y_i - \bar{y})^2$ represents the goodness of fit (Kvålseth, 1985; Tzafriri and Edelman, 2004, 2005). Here y_i are the data points extracted from the full system (10), $\bar{y}$ is the average of y_i and $\hat{y}_i$ is the fitted (approximated) value corresponding to y_i for each i. $R^2 = 1$ for a perfect fit, lower R^2 value indicates a worse fit, and $R^2 < 0$ represents that the constant $\bar{y}$ is a better fit than the approximating curve. It should be remarked that R^2 values must be used with great care for nonlinear models, and that there exist several definitions of the R^2 value. However, the definition applied here seems to be preferable (Kvålseth, 1985).

Figure 4A shows the fractional errors for different enzyme to substrate ratios E_0/S_0, $S_0 = S_{1,0} = S_{2,0}$ and for comparable affinities. Our competitive tQSSA (21) gives very low errors for the full range of ratios, and is consistently better than all the other approximations. The figure also shows that the first-order approximation (49) is a decent fit for all values of E_0/S_0, but that it is inferior to the competitive sQSSA (13) at low enzyme-to-substrate ratios, and to the single-reaction tQSSA (5) at high ratios. Hence, the advantage of the first-order approximation (49) is that we have an approximation giving reasonable predictions for a wider range of parameters, rather than an approximation, which is more accurate than the best of the competitive sQSSA (13) and the single-reaction tQSSA (5).

When varying the ratio of the affinities $K_M^{(1)}/K_M^{(2)}$, we obtain again that the competitive tQSSA (21) is an excellent approximation for all values of this ratio (Fig. 4B), and it is again superior to the other approximations. The fractional errors associated with both the competitive sQSSA (13) and the single-reaction tQSSA (5) are almost unchanged for different $K_M^{(1)}/K_M^{(2)}$ ratios, and for low ratios they are both comparable to the error related to the first-order approximation

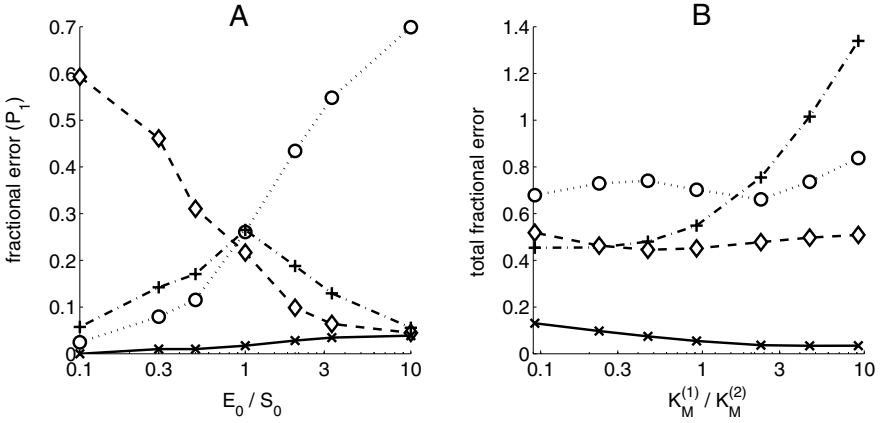

Fig. 4 The fractional errors associated with the different approximations. Panel A shows the effect of varying the enzyme-to-substrate ratio E_0/S_0, where $S_0 = S_{1,0} = S_{2,0}$, measured by the fractional errors of P_1. Panel B shows the total fractional error, i.e, for the sum of the fractional errors for P_1 and P_2, when varying $K_M^{(2)}$ reflected in the ratio $K_M^{(1)}/K_M^{(2)}$. Line styles correspond to Fig. 1 with markers: Competitive tQSSA (21) (×); competitive sQSSA (13) (○); single-reaction tQSSA (5) (◇) and first-order approximation (49) (+). Parameters are as in Fig. 1 except: Panel A: $S_0 = 5, 15, 25\ \mu$M for $E_0/S_0 > 1$ and $E_0 = 5, 15, 25\ \mu$M for $E_0/S_0 < 1$. Panel B: $K_M^{(2)} = 2.5, 5, 10, 25, 50, 100, 250\ \mu$M. The points in panel A for $E_0/S_0 = 0.1, 1$, and 10 correspond to Figs. 1, 2A, and 2B, respectively. The case $K_M^{(2)} = 25\ \mu$M ($K_M^{(1)}/K_M^{(2)} \approx 1$) correspond to Fig. 1.

(49). For high values of $K_M^{(1)}/K_M^{(2)}$, or more precisely low $K_M^{(2)}$ values, the first-order approximation breaks apparently down. This is due to large fractional errors with respect to P_1, and is related to the large value of $\tilde{K}_{M,0}^{(1)}$ compared to $K_M^{(1)}$. This is not the case for the low $K_M^{(1)}/K_M^{(2)}$ ratio in Fig. 4B, since these are for high $K_M^{(2)}$ values and since $K_M^{(1)}$ and $S_{1,0}$ are of the same magnitude, and hence $\tilde{K}_{M,0}^{(2)}$ and $K_M^{(2)}$ are of similar magnitude.

In the special case of identical affinities we saw that our approach should be at least roughly valid if only $K \leq K_M$. This last assumption seems to be reasonable, since if $K_M^{(1)} \approx K_M^{(2)}$, we expect that the kinetic parameters $k_1^{(i)}$ and $k_2^{(i)}$ are similar for the two substrates. This would imply $K \approx k_2^{(i)}/k_1^{(i)}$ for the same i and consequently $K \lessapprox K_M$. Interestingly, for many metabolic enzymes $k_2 \ll k_{-1}$, i.e., $K \ll K_M$ (Atkinson, 1977). This implies that for competing substrates with identical affinities the relation $K \leq K_M$ is even more reasonable, and even $K \ll K_M$ can be expected, in which case the tQSSA (21) is a very good approximation, as seen from (30). Based on the above mentioned fact that often $k_2^{(i)} \ll k_{-1}^{(i)}$, Bhalla and Iyengar (1999) use the relation $k_{-1}^{(i)} = 4k_2^{(i)}$. Then for identical affinities $k_1^{(i)} = 5k_2^{(i)}/K_M^{(i)} = 5k_2^{(i)}/K_M$, such that

$$K = \frac{\max_i k_2^{(i)}}{\min_i k_1^{(i)}} = \frac{\max_i k_2^{(i)}}{(5/K_M)\min_i k_2^{(i)}} = \frac{K_M}{5}\frac{\max_i k_2^{(i)}}{\min_i k_2^{(i)}},$$

from which it is seen that $K \leq K_{\mathrm{M}}$ unless $k_2^{(1)}$ and $k_2^{(2)}$ differ by more than a factor 5. This is not the case for any of the IKK-2 data with $K_{\mathrm{M}}^{(1)} \approx K_{\mathrm{M}}^{(2)}$ from Kishore et al. (2003), nor for carbohydrate sulfotransferase (NodST) with chitotriose and chitopentaose as substrates (Pi and Leary, 2004). In fact, their k_{cat} (our $k_2^{(i)}$) values differ by less than a factor 2.

The assumption $K \leq K_{\mathrm{M}}^{(i)}$ cannot be expected to hold when $K_{\mathrm{M}}^{(1)} \ll K_{\mathrm{M}}^{(2)}$, as illustrated by Fig. 3. Assuming again $k_{-1}^{(i)} = 4k_2^{(i)}$, such that $k_1^{(i)} = 5k_2^{(i)}/K_{\mathrm{M}}^{(i)}$, then gives

$$K = \frac{\max_i k_2^{(i)}}{\min_i k_1^{(i)}} = \frac{\max_i k_2^{(i)}}{5 \min_i k_2^{(i)}/K_{\mathrm{M}}^{(i)}} \leq \frac{K_{\mathrm{M}}^{(2)}}{5} \frac{\max_i k_2^{(i)}}{\min_i k_2^{(i)}},$$

so again $K \leq K_{\mathrm{M}}^{(2)}$ unless $k_2^{(1)}$ and $k_2^{(2)}$ differ by more than a factor 5. On the other hand,

$$K = \frac{\max_i k_2^{(i)}}{5 \min_i k_2^{(i)}/K_{\mathrm{M}}^{(i)}} \geq \frac{\max_i k_2^{(i)}}{5(\max_i k_2^{(i)})(\min_i 1/K_{\mathrm{M}}^{(i)})} = \frac{K_{\mathrm{M}}^{(2)}}{5},$$

such that if $K_{\mathrm{M}}^{(2)} > 5K_{\mathrm{M}}^{(1)}$ we will have $K > K_{\mathrm{M}}^{(1)}$. The parameters in Fig. 3 are such that $K_{\mathrm{M}}^{(1)} < K < K_{\mathrm{M}}^{(2)}$.

Related to the condition $K \leq K_{\mathrm{M}}^{(i)}$ is the difference between ϵ from (26) and $\bar{\epsilon}$ from (27), which can be significant. In Fig. 5 the (inaccurate) expression from (27) gives $\bar{\epsilon} = 0.0580$, which is lower than ϵ in Fig. 2B where the tQSSA (21) is a reasonable approximation. But in Fig. 5 the tQSSA (21) does not fit as well as in the previous figures ($R^2 = 0.9907$), and indeed the correct formula from (26) gives a significantly higher value $\epsilon = 0.1005$.

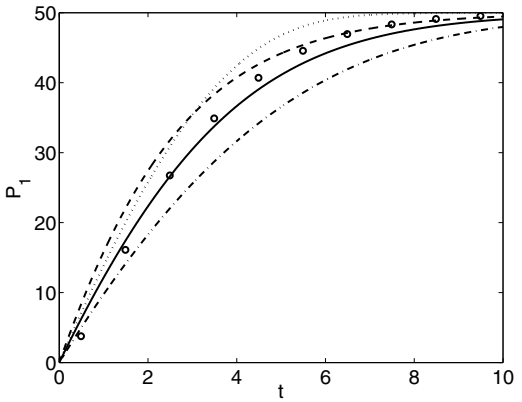

Fig. 5 When ϵ becomes large also the tQSSA (21) fails. Parameters and legends are as in Fig. 1, but with the constraint $k_{-1}^{(i)} = 0.1k_2^{(i)}$, such that $K = 38.18 > K_{\mathrm{M}}^{(i)}$, to force a large $\epsilon = 0.1005$ ($\bar{\epsilon} = 0.0580$ from (27)). R^2 values: [0.9907, 0.9555, 0.9568, 0.9119].

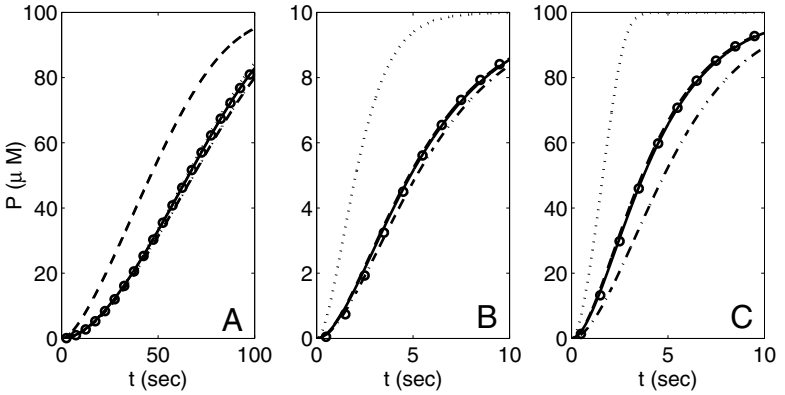

Fig. 6 The tQSSA (21) estimates the development of the product of two successive reactions catalyzed by the same enzyme well, and the discussion of the validity of the sQSSA (13), the single-reaction tQSSA (5) and the first-order tQSSA (49) apparently carries over to this case. Legends are as in Fig. 1, and parameters as in Fig. 2 except for the initial substrate concentrations, which are $S_{2,0} = 0$ in all panels and: In A: $S_{1,0} = 100$ ($R^2 = [1.0000, 0.9988, 0.5759, 0.9949]$). In B: $S_{1,0} = 10$ ($R^2 = [0.9984, -0.3307, 0.9973, 0.9931]$). $E_0 = 5$. $\epsilon = 0.0832$. In C: $S_{1,0} = 100$ ($R^2 = [0.9997, -0.0351, 0.9986, 0.9076]$).

Our results are immediately applicable to, e.g., successive reactions catalyzed by the same enzyme, such as nonprocessive or distributive double phosphorylation or dephosphorylation processes, as seen for example in the MAPK cascade (Burack and Sturgill, 1997; Ferrell and Bhatt, 1997; Zhao and Zhang, 2001; Markevich et al., 2004). The reaction scheme can be seen as a special case of (2) with $P_1 = S_2$ and is summarized as

$$S_1 \xrightarrow{E} S_2 \xrightarrow{E} P,$$

where it is usually assumed that at the beginning only S_1 is present. Figure 6 shows that the results presented here yield a good approximation ($R^2 > 0.998$ in the three examples). This is in great contrast to the competitive sQSSA (13), which in both panels B and C of Fig. 6 gives negative R^2 values.

However, it should be remarked that our theoretical investigation of the validity of the tQSSA does not work in the case of successive reactions. The problem is that there is no S_2 at time $t = 0$, and hence the timescales cannot be found following Segel (1988) because the definition of the transient phase no longer holds. Nevertheless, it seems like the conclusions concerning the validity of the first-order approximation from above carry over to this scenario (compare the three panels of Fig. 2 with the panels of Fig. 6). We will present the investigation of such reactions in another paper.

Finding approximations extending the classical sQSSA approach for complex reactions such as successive reactions, open systems, loops such as the Goldbeter and Koshland switch (Goldbeter and Koshland, 1981), feedback systems, etc. should be of great interest for further improving investigations and simulations of such reactions in vivo, where the sQSSA description breaks down. The alternative is to

simulate each step of the reaction, i.e., the full system of ODEs, but for larger systems this can quickly become very computer expensive. Moreover, all of the (often unknown) kinetic parameters are needed for a full simulation, while a QSSA usually needs only K_M and V_{max} values. Furthermore, the QSSA can provide theoretical insight which is hard to gain from the full system, for example in the way the classical sQSSA (3) explains the saturation curve. We expect that the ideas presented here can be used to extend the tQSSA to the above (and, hopefully, other and more complex) reactions.

Acknowledgements

The authors are deeply grateful to the anonymous referees, who gave much appreciated suggestions to several passages of the present paper, which helped clarifying the manuscript greatly. Dr. Giuliana Cortese gave precious suggestions and considerations related to the usage of R^2 values. M.G.P. was supported by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137.

Appendix A: Existence and uniqueness of the solution for the complexes

We show the existence and uniqueness of a solution to the system (18) with $0 < C_i < \min\{\bar{S}_i, E_0\}$. First we note that (18) implies

$$\frac{K_M^{(1)} C_1}{\bar{S}_1 - C_1} = \frac{K_M^{(2)} C_2}{\bar{S}_2 - C_2},$$

from which it is seen that $0 < C_1 < \bar{S}_1$ if and only if $0 < C_2 < \bar{S}_2$.

Substituting (18b) into (18a) leads to the following equation in C_1

$$C_1 = E_0 - \left(E_0 - C_1\left(1 + \frac{K_M^{(1)}}{\bar{S}_1 - C_1}\right)\right)\left(1 + \frac{K_M^{(2)}}{\bar{S}_2 - \left(E_0 - C_1\left(1 + \frac{K_M^{(1)}}{\bar{S}_1 - C_1}\right)\right)}\right) \quad \text{(A.1)}$$

and C_2 can then be found from (18b).

Solving (A.1) is equivalent to finding roots of the third-degree polynomial

$$\begin{aligned}\psi_1(C_1) = &-\left(K_M^{(1)} - K_M^{(2)}\right)C_1^3 \\ &+\left[\left(E_0 + K_M^{(1)} + \bar{S}_1\right)\left(K_M^{(1)} - K_M^{(2)}\right) - \left(\bar{S}_1 K_M^{(2)} + \bar{S}_2 K_M^{(1)}\right)\right]C_1^2 \\ &+\left[-E_0\left(K_M^{(1)} - K_M^{(2)}\right) + \left(\bar{S}_1 K_M^{(2)} + \bar{S}_2 K_M^{(1)}\right) + K_M^{(2)}\left(E_0 + K_M^{(1)}\right)\right]\bar{S}_1 C_1 \\ &- E_0 K_M^{(2)} \bar{S}_1^2. \end{aligned} \quad \text{(A.2)}$$

An analogous polynomial ψ_2 for C_2 can be found by interchanging the indexes 1 and 2 in (A.2), because of the symmetry of the system (17). Rearranging the terms, ψ_1 can also be written

$$\begin{aligned}\psi_1(C_1) = {} & K_{\mathrm{M}}^{(2)}(C_1 - E_0)(\bar{S}_1 - C_1)^2 \\ & + K_{\mathrm{M}}^{(1)} C_1 \big(C_1 + K_{\mathrm{M}}^{(2)} + \bar{S}_2 - E_0\big)(\bar{S}_1 - C_1) + \big(K_{\mathrm{M}}^{(1)} C_1\big)^2. \end{aligned} \tag{A.3}$$

From (A.2) we see that $\psi_1(0) < 0$, and from (A.3) that $\psi_1(\bar{S}_1) > 0$. Hence, ψ_1 has at least one root between 0 and $\bar{S}_1$, which shows existence.

When $K_{\mathrm{M}}^{(1)} \neq K_{\mathrm{M}}^{(2)}$, we can without loss of generality assume that $K_{\mathrm{M}}^{(1)} > K_{\mathrm{M}}^{(2)}$ because of the symmetry of (2). In this case $\lim_{c\to\pm\infty} \psi_1(c) = \mp\infty$, and we see that ψ_1 has one negative root and one root larger than $\bar{S}_1$. Hence, there is a unique root $C_1 \in (0, \bar{S}_1)$, which also solves (A.1). This implies the uniqueness of the solution.

When $K_{\mathrm{M}}^{(1)} = K_{\mathrm{M}}^{(2)} = K_{\mathrm{M}}$, ψ_1 becomes a second-degree polynomial. Because of (A.2) we have $\lim_{c\to\infty} \psi_1(c) = -\infty$, so the second root is larger than $\bar{S}_1$. Hence, also in this case we have only one root between 0 and $\bar{S}_1$, given by (20).

The approach to solving (18) taken above helps the theoretical reasoning, but is practically cumbersome, since we need to find the largest $K_{\mathrm{M}}^{(i)}$. In addition, the formula (18b) for finding C_2 is numerically imprecise when both C_1 and $\bar{S}_1$ are small. Both these problems can be overcome by finding the root of the polynomial ψ_2 for C_2; ψ_2 has a single root in $(0, \bar{S}_2)$ as a consequence of the uniqueness result.

Appendix B: Validity of the first-order approximation of the root of ψ_i

To investigate the validity of (49) we evaluate ψ_1 from (19) at C_1 given by (48) This yields the remainder

$$\begin{aligned} R_1 := \psi_1(C_1) = {} & -E_0^2 \bar{S}_1^2 K_{\mathrm{M}}^{(2)} \big[K_{\mathrm{M}}^{(2)}\big(E_0 + K_{\mathrm{M}}^{(1)}\big) + K_{\mathrm{M}}^{(2)} \bar{S}_1 + K_{\mathrm{M}}^{(1)} \bar{S}_2\big]^{-3} \\ & \times \big[K_{\mathrm{M}}^{(1)} K_{\mathrm{M}}^{(2)} \big(\bar{S}_1 \bar{S}_2 \big(K_{\mathrm{M}}^{(1)} + K_{\mathrm{M}}^{(2)}\big) + E_0 \big(\bar{S}_1 K_{\mathrm{M}}^{(2)} + \bar{S}_2 K_{\mathrm{M}}^{(1)}\big)\big) \\ & + \bar{S}_1 \big(K_{\mathrm{M}}^{(2)}\big)^3 \big(\bar{S}_1 + K_{\mathrm{M}}^{(1)}\big) + \bar{S}_2 \big(K_{\mathrm{M}}^{(1)}\big)^3 \big(\bar{S}_2 + K_{\mathrm{M}}^{(2)}\big)\big]. \end{aligned} \tag{B.1}$$

The term "remainder" is used, since if R_1 were zero, then C_1 given by (48) would be a true root, not only an approximation. To have a good approximation of the true root, $|R_1|$ must be small compared to typical sizes of ψ_1 such as

$$|\psi_1(0)| = E_0 K_{\mathrm{M}}^{(2)} \bar{S}_1^2 \quad \text{and} \quad \psi_1(\bar{S}_1) = \big(K_{\mathrm{M}}^{(1)} \bar{S}_1\big)^2.$$

Similar conditions should hold for C_2 and ψ_2, but calculations and results are identical, and we show them only for C_1 in the following.

When (14) holds we expect (49) to hold, and then it reduces to the sQSSA (13). In this case the terms involving E_0 in R_1 are negligible and the condition $|R_1| \ll |\psi_1(0)|$ implies

$$\frac{E_0}{(\bar{S}_1 + \tilde{K}_{\mathrm{M}}^{(1)})^2} \times \frac{K_{\mathrm{M}}^{(1)}}{K_{\mathrm{M}}^{(2)}} \left(\bar{S}_2 \frac{K_{\mathrm{M}}^{(1)}}{K_{\mathrm{M}}^{(2)}} + \bar{S}_1 \frac{\bar{S}_2 + \tilde{K}_{\mathrm{M}}^{(2)}}{\bar{S}_1 + \tilde{K}_{\mathrm{M}}^{(1)}} \right) \ll 1, \tag{B.2}$$

where we have introduced the so-called apparent Michaelis constants (see, e.g., Schnell and Mendoza (2000))

$$\tilde{K}_{\mathrm{M}}^{(i)} = K_{\mathrm{M}}^{(i)} \left(1 + \bar{S}_j / K_{\mathrm{M}}^{(j)}\right), \quad j \neq i. \tag{B.3}$$

Similarly $|R_1| \ll \psi_1(S_1)$ can be restated as

$$\left(\frac{E_0}{\bar{S}_1 + \tilde{K}_{\mathrm{M}}^{(1)}} \right)^2 \left(\frac{\bar{S}_2}{K_{\mathrm{M}}^{(2)}} + \frac{\bar{S}_1}{K_{\mathrm{M}}^{(1)}} \frac{\bar{S}_2 + \tilde{K}_{\mathrm{M}}^{(2)}}{\bar{S}_1 + \tilde{K}_{\mathrm{M}}^{(1)}} \right) \ll 1. \tag{B.4}$$

These conditions are both clearly satisfied by (14) as long as $\bar{S}_i$ is not much greater than $K_{\mathrm{M}}^{(i)}$, and $K_{\mathrm{M}}^{(1)}$ and $K_{\mathrm{M}}^{(2)}$ are of similar magnitude.

At high enzyme concentrations, (49) is a good approximation whenever $K_{\mathrm{M}}^{(1)} \approx K_{\mathrm{M}}^{(2)}$, as stated in (35), which stimulates the assumption

$$E_0 + K_{\mathrm{M}}^{(i)} \gg S_{1,0} + S_{2,0}, \quad i = 1, 2. \tag{B.5}$$

Our condition $|R_1| \ll |\psi_1(0)|$ then becomes

$$\frac{E_0}{(E_0 + \tilde{K}_{\mathrm{M}}^{(1)})^2} \times \frac{K_{\mathrm{M}}^{(1)}}{K_{\mathrm{M}}^{(2)}} \left(\bar{S}_2 \frac{K_{\mathrm{M}}^{(1)}}{K_{\mathrm{M}}^{(2)}} + \bar{S}_1 \frac{E_0 + \tilde{K}_{\mathrm{M}}^{(2)}}{E_0 + \tilde{K}_{\mathrm{M}}^{(1)}} \right) \ll 1, \tag{B.6}$$

which is guaranteed by (B.5) if $K_{\mathrm{M}}^{(1)}$ and $K_{\mathrm{M}}^{(2)}$ are of similar magnitude.

The other condition, $|R_1| \ll \psi_1(S_1)$, is now

$$\left(\frac{E_0}{E_0 + \tilde{K}_{\mathrm{M}}^{(1)}} \right)^2 \left(\frac{\bar{S}_2}{K_{\mathrm{M}}^{(2)}} + \frac{\bar{S}_1}{K_{\mathrm{M}}^{(1)}} \frac{E_0 + \tilde{K}_{\mathrm{M}}^{(2)}}{E_0 + \tilde{K}_{\mathrm{M}}^{(1)}} \right) \ll 1. \tag{B.7}$$

This is, on the other hand, not guaranteed by (B.5); we must require, for example, that

$$K_{\mathrm{M}}^{(i)} \gg S_{i,0}. \tag{B.8}$$

Then (49) reduces to the single-reaction first-order tQSSA (9).

At high enzyme concentrations, but low $\bar{S}_i$ and $K_{\mathrm{M}}^{(i)}$ values, we can estimate the error that we make by using the first-order tQSSA. The remainder R_1 from (B.1) is negative, which implies that (48) is an underestimate. The relative error err_{rel},

given as the actual error $err \leq \bar{S}_1 - C_1$ divided by the maximal possible error $\bar{S}_1$, is then bounded by

$$err_{\text{rel}} \leq \frac{\bar{S}_1 - C_1}{\bar{S}_1} = \frac{K_{\text{M}}^{(1)}(1 + \bar{S}_2/K_{\text{M}}^{(2)}) + \bar{S}_1}{K_{\text{M}}^{(1)}(1 + \bar{S}_2/K_{\text{M}}^{(2)}) + \bar{S}_1 + E_0} \leq \frac{\tilde{K}_{\text{M},0}^{(1)} + S_{1,0}}{\tilde{K}_{\text{M},0}^{(1)} + S_{1,0} + E_0},$$

which is indeed small for large E_0, say

$$E_0 \gg \tilde{K}_{\text{M},0}^{(i)} + S_{i,0}, \quad i = 1, 2. \tag{B.9}$$

Hence, only for an intermediate range of large, but not too large, values of E_0 is the first-order approximation bad. When $K_{\text{M}}^{(1)} \approx K_{\text{M}}^{(2)}$, we can use (B.5) instead of (B.8) or (B.9) as a criterion for the first-order approximation to be near the full tQSSA in agreement with (35).

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